DISCOVERY

58(322), October 2022

Phytochemical, antioxidant and antibacterial activities of seed kernel extracts of *Mangifera indica* Linn. varieties

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To Cite:
Nwachukwu KC, Ugbogu OC. Phytochemical, antioxidant and antibacterial activities of seed kernel extracts of *Mangifera indica* Linn. varieties. *Discovery*, 2022, 58(322), 1054-1063

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Peer-Review History
Received: 31 July 2022
Reviewed & Revised: 05/August/2022 to 06/September/2022
Accepted: 09 September 2022
Published: October 2022

Peer-Review Model
External peer-review was done through double-blind method.

ABSTRACT

The aim of the research was to determine new antibacterial agents with antioxidant and phytochemical properties. The phytochemicals contents and antioxidant activities of the varieties were quantitatively determined with spectrophotometer. Antibacterial activities of the *Opioro* and *Julie* extracts were performed using microdilution assay. From the result, the total phenolics, total tannins, total flavonoids, total saponins and total alkaloids were significantly higher in *Julie* than *Opioro*. *Julie* also had higher ABTS^{*+} scavenging activity and reducing powers while DPPH* scavenging activity did not vary significantly between the two varieties. All the typed strains were susceptible to the extracts of the two varieties. The lowest minimum inhibitory concentration of 31.25 µg/ml and 125 µg/ml for acetone and ethanolic extracts of *Opioro* respectively were observed against *Staphylococcus aureus* ATCC 6538. While the *Julie* variety produced low minimum inhibitory concentration (MIC) against *E. coli* O157 at 7.18 µg/ml (acetone extract) and 62.25 µg/ml (ethanolic extract). The findings from this study suggest that the seed kernel extracts, especially *Julie* had rich phytochemicals and antioxidant properties and may be good candidates for drug development.

Key words: Seed kernels, Mango varieties, antibacterial, phytochemical, antioxidant

1. INTRODUCTION

Since antiquity, medicinal plants have been used for the treatment of infectious diseases due to their therapeutic characteristics; contributing to these effects is their ability to synthesize secondary bioactive components (Hao and Xiao, 2020). These bioactive compounds have been identified as essential agents in development of drugs (Pan et al., 2013). They have been modified by some pharmaceutical companies, hitherto to improve the quality of herbal medicine. Atanasov et al. (2015) discovered several factors that had led to reduction in use of plants as agents for drug development. The inability to access the plant source, unavailability of the plant components, complexity of the chemical products, lack of proper genetic mode of action, environmental and government restrictions on

the use of such plants, uncontrollable use and foraging of the plants and laborious processes of clinical trials to ascertain their efficacy are some of the few challenges outlined by Atanasov et al. (2015). The afore-mentioned challenges have lowered the morale of most drug-producing companies in the use of plants as model for new drug development. Despite the shortcomings, medicinal plants are still considered the most efficient therapeutic agents when compared with chemical drugs (Pan et al., 2013).

Mangifera indica L. belongs to the family of Anacardiaceae, one of the major perennial fruit plants cultivated world-wide especially in tropical areas. It is known several years ago to be therapeutically active in traditional medicine (Tollenaere et al., 2022; Gautama Reddy et al., 2016). The leaves, stem bark, fruits, and roots are essential in the treatment of inflammation, constipation, cough, diarrhoea, cancer and several diseases caused by viruses, bacteria and parasites (Parvez, 2016). These therapeutic properties are as a result of phytochemicals they possess (Batool et al., 2018; Lauricella et al., 2017).

The seed kernel extracts of *M. indica* L. have been neglected, but recently, they have been discovered to exhibit strong pharmacological and nutritional properties contributed by their rich bioactive chemicals (Mwaurah et al., 2020; Kittiphoom, 2012).

There are reports on the use of seed kernels as food supplements for humans and feeds for livestock; their methanolic, acetone and ethanolic extracts are known to be curative agents for diabetes, hypertension, inflammation and diarrhoea in animal models (Torres-Leon et al., 2017).

M. indica L. seed kernels extracts exhibited strong antibacterial activity against pathogenic Gram positive and Gram-negative bacteria *in vivo* (Kaur et al., 2010). They are effective in the treatment of fungal and viral infections. Meanwhile, only few scientific research reported the phytochemical contents, antioxidants, and antibacterial properties of seed kernels of some varieties of mango such as *Elemi*, *Sherri*, *Ogbomoso* (Irondi et al., 2018); *Kent*, *Ngowe*, *Apple* and *Sabine* (Mutua et al., 2016) but, no scientific paper had a report for those of *Opioro* and *Julie* varieties. Hence, the present work is particularly aimed at closing the gap in the discovery of new drug capable of inhibiting bacteria growth while decreasing oxidative reactions with little or no side effects.

2. MATERIALS AND METHODS

2.1. Sample collection

The two varieties (*Opioro* and *Julie*) of *Mangifera indica* L. used for this study were obtained from Umuagbaghi village in Aba South LGA of Abia state. The taxonomist, Mrs. Chikodiri from the Department of Plant Sciences and Biotechnology, Abia State University, Uturu identified the samples with their leaves and fruits. Voucher specimens of *Opioro* (ABSU/PSB/250) and *Julie* (ABSU/PSB/251) varieties were deposited at the herbarium of the department. The fruits of these varieties were transferred to the laboratory for processing.

2.2. Sample preparation and processing

The fleshy part of *Opioro* and *Julie* mangoes was separated from the seed kernel using sharp knife and the seed kernel in turn was cut in pieces and air-dried under shade at room temperature for 4 weeks and later milled into powder using Panasonic electric blender (MX-AC210 model, 170 mm x 236 mm x 268 mm Panasonic, Japan). The powdered samples were kept in airtight container and stored at 4^{o}C in a refrigerator (Ahmed et al., 2007).

2.2.1. Preparation of acetone extracts of mango seed kernels

Fifty grams of the milled seed kernel was mixed in 500 ml of 85% acetone for 12 h. The mixture was stirred for about 4 h at room temperature. Then the extract was filtered using Whatman No. 1 filter paper to separate the residue from the filtrate. The filtrate was dried at 40^{o}C using water bath to obtain concentrated dried extracts in powder form. The extract was stored at -20^{o}C for subsequent use (Yousef et al., 2009).

2.2.2. Preparation of ethanolic Extracts of Mango Seed Kernels

Fifty grams of the milled seed kernel was mixed in 500 ml of 70% ethanol for 12 h. Then the extract was filtered through Whatman No. 1 filter paper. The supernatant was evaporated using the water bath at 25^{o}C to remove the ethanol and obtain concentrated extracts. It was stored at -20^{o}C for subsequent use (Yousef et al., 2009).

2.3. Phytochemical analysis

The total phenolics, total saponins, total tannins, total flavonoids and total alkaloids of *Mangifera indica* seed kernel were quantitatively determined using the procedure of Gupta et al. (2013).

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2.4. Estimation of DPPH* free-radical-scavenging ability of *Mangifera indica* seed kernels

The modified protocol of Sharma and Bhat (2009) was used to estimate the free-radical-scavenging ability of *Opioro* and *Julie* extracts of *Mangifera indica* L. seed kernel against 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical. Briefly, 1.0 ml dilution of the extracts was mixed with 3.0 ml of 60µM methanolic solution of DPPH radicals; and the mixture was left in the dark for 30 min before the absorbance was taken at 517 nm. The decrease in absorbance of $DPPH^{\cdot}$ on addition of test samples in relation to the control was used to calculate the percentage inhibition following the equation:

$$\% \text{ Inhibition} = A_{control} - A_{Sample} / A_{control} \times 100$$

2.5. Estimation of ABTS*+ radical-scavenging ability of Mangifera indica L. seed kernel

The $ABTS^{*+}$ radical-scavenging ability of *Opioro* and *Julie* extracts were determined in accordance with the method described by Sellappan et al. (2002). The $ABTS^{*+}$ radical was generated by incubating equal volume of a 7 mM $ABTS^{*+}$ aqueous solution with $K_2S_2O_8$ (2.45 mM) in the dark for 16 h at room temperature and adjusting the absorbance at 734 nm to 0.7 ± 0.02 with 95% ethanol. Then 0.2 ml of the diluted extract was added to 2.0 ml ABTS* solution and the absorbance was measured at 734 nm after 15 min. The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated. The calibration equation for TEAC was $Y = -0.0505x + 0.1954$ ($R^2 = 0.9902$).

2.6. Estimation of reducing power

The ability of the *Opioro* and *Julie* extracts to reduce $FeCl_3$ solution was evaluated according to the protocol of described by Oyaizu (1986). Briefly, 2.5 ml aliquot of each extract was mixed with 2.5 ml of 200 mM sodium phosphate buffer at pH of 6.6 and 2.5 ml of 1% potassium ferricyanide. After 20 min of incubation of the mixture at 50°C, 2.5 ml of 10% trichloroacetic acid was added. This mixture was divided into aliquots of 2.5 ml in different test tubes, and each portion was diluted with 2.5 ml of distilled water. Next, 1 ml of 0.1% ferric chloride was added to each tube. The absorbance was measured at 700 nm, and the reducing power of the extracts was subsequently calculated using gallic acid standard curve.

2.7. Bacterial strains

The four selected strains, *Escherichia coli* O157, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, used for antibacterial study were obtained from National Veterinary Research Institute, Vom, Plateau state, Nigeria.

2.8. Preparation of Bacterial strains

2.8.1. Reviving of lyophilized strain

The outer surface of the sealed lid was properly disinfected by cleaning it with 70% ethanol; and allowed to dry. The lid was aseptically removed afterwards and two drops of sterile water was pipetted into the walls of container to decrease adherence of the pellets. The pellets were suspended into 5 ml of peptone water in sterile test tubes, properly mixed with vortex mixer and allowed for 1 hour on work bench before incubating at 37°C for 48 hours. A loopful of the suspension was transferred to already prepared NA plates by streaking and incubated overnight at 37°C for possible colonies of pure culture. Furthermore, a colony from the agar plates was sub-cultured onto agar slant and was later incubated at 37°C for 24 h for storage.

2.8.2. Preparation of strains for Antibacterial activity

The bacteria strains suspensions were prepared by aseptically transferring a loopful of colony of the strains from each of the freshly prepared agar slant into Eppendorf tubes containing Mueller-Hinton and properly mixed with sterile glass rod. The culture suspensions in each of the Eppendorf tube were adjusted by comparing against 0.5 McFarland turbidity standard tubes. One hundred microliter of the suspension was transferred aseptically and appropriately into the wells of microplate during antimicrobial activity test.

2.9. Antibacterial test

Antibacterial activity of the extracts on the bacterial strains was evaluated using the microdilution broth method (Eloff, 2019) with slight modification. A stock solution of acetone and ethanolic extracts of *Opioro* and *Julie* varieties was prepared separately by dissolving 500 mg of each extract in 1 ml of normal saline to reconstitute them. Briefly, 100 µl of distilled water was transferred into the wells of 96 microtitre plates using multichannel micropipette. The first well was taken as the control. Exactly 100 µl of 500 mg/ml from the stock was transferred to the second well and the content mixed properly by pipetting the well up and down a few

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times. Another 100 µl was transferred from the second well to the third well and mixed properly. The two-fold dilution process was repeated until the last well where 100 µl was discarded. Then, 100 µl of the adjusted bacterial strain (10^6 cfu/ml), *E. coli* O157, in Mueller-Hinton broth was added to the negative control well and the other wells. The experiment was repeated in triplicate. The microtitre plate was properly covered with the lid and incubated at 37°C for 24 h. Thereafter, the absorbance was determined using microplate reader (ELx808™ Biotek, Britain) at wavelength of 620 nm. For each concentration, the absorbance level in the experiment was converted to percentage growth. Therefore, percentage growth of each bacterial strain was calculated with the formula: Percentage growth = absorbance value of each bacterial strain/negative control absorbance value of each bacterial strain×100

Control

Negative control comprised of 100 µl of distilled water

2.10. Minimum inhibitory concentration analysis

The minimum inhibitory concentration of each extract was subsequently determined by comparing the wells with the least absorbance values for each extract concentration.

2.11. Statistical analysis

The results were expressed as means±SD using graph pad prism graphical statistical package version 5. The student t-test at $p<0.05$ was applied to assess the difference between the mean for two variables and two-way analysis of variance (ANOVA), followed by Bonferreni post hoc test.

3. RESULTS

3.1. **Phytochemical analysis**

The quantitative screening of phytochemical constituents in the two varieties (*Opioro* and *Julie*) of mango seed kernel in Table 1 indicates appreciable amount of total phenolics, total saponins, flavonoids, total tannins and alkaloids. Total phenolics, total flavonoids, total tannins, total saponins and total alkaloids were significantly ($p<0.05$) higher in *Julie* than *Opioro*.

Table 1: The phytochemical compositions of seed kernel extracts of *Opioro* and *Julie* varieties.

Phytochemicals	Seed kernel varieties		P value
	Opioro (mg/g)	*Julie* (mg/g)	
Total phenolics	51.245±0.39[a]	95.135±0.32[b]	P<0.001
Flavonoids	17.490±0.23[a]	19.195±0.06[b]	P<0.01
Tannins	45.275±±0.26[a]	71.805±0.33[b]	P<0.001
Total saponins	47.53±0.28[a]	56.525±0.50[b]	P<0.001
Total alkaloids	22.990±0.17[a]	27.595±0.09[b]	P<0.001

Values in the figure were analysed with two-way ANOVA and then expressed as mean ± standard deviation (SD) of two replicates. Along the same row, values that have different superscript alphabet vary significantly.

3.2. Antioxidant

The antioxidant activities of *Opioro* and *Julie* varieties are presented in Table 2. The result showed that the ABTS scavenging ability and the reducing powers of *Julie* were significantly higher than *Opioro* ($p<0.05$). The DPPH scavenging abilities of both *Opioro* and *Julie* were not significantly different ($p>0.05$).

3.3. Antibacterial activity

The concentration-dependent curve of antimicrobial activities of acetone and ethanolic extracts of *Opioro* and *Julie* varieties on different strains of Gram-positive and Gram-negative bacterial typed strains are shown in Figures 1–4. Each extract was treated against *E. coli* O157, *P. aeruginosa* ATCC 9027, *S. aureus* ATCC 6538 and *B. subtilis* ATCC 6633. All the typed stains were susceptible to the extracts as they showed a positive decline from the control.

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Table 2: DPPH* SC_{50}, ABTS*+ scavenging abilities and reducing power of seed kernel extracts of two varieties of *Mangifera indica* L. on dry weight basis.

Mangifera indica L. Variety	DPPH* SC_{50} (mg/g)	ABTS* Scavenging ability (µmol TEAC/g)	Reducing power (mg GAEC/g)
Opioro	0.3150±0.007071[a]	3280.50±4.681[a]	63.82±0.453[a]
Julie	0.2850±0.007071[a]	4824.95±2.008[b]	72.24±0.537[b]

Values in the figure were analysed with two-way ANOVA and then expressed as mean±standard deviation (SD) of two replicates. Values having different superscript alphabets along the column vary significantly (P<0.05).

Key: SC_{50}: extract concentration that scavenged 50% of DPPH; TEAC: Trolox equivalent antioxidant capacity; GAE: Gallic acid equivalent.

Table 3: Minimum Inhibitory Concentration (MIC) (µg/ml) of Acetone and Ethanolic extracts of *Opioro* and *Julie*

	Opioro Extract (µg/ml)		Julie Extract (µg/ml)	
Organism	**Acetone**	**Ethanol**	**Acetone**	**Ethanol**
E. coli O157	62.25	125	7.81	62.25
P. aeruginosa ATCC9027	62.25	125	62.25	62.25
S. aureus ATCC 6538	31.25	125	62.25	125
B. subtilis ATCC 6633	250	125	31.25	62.25

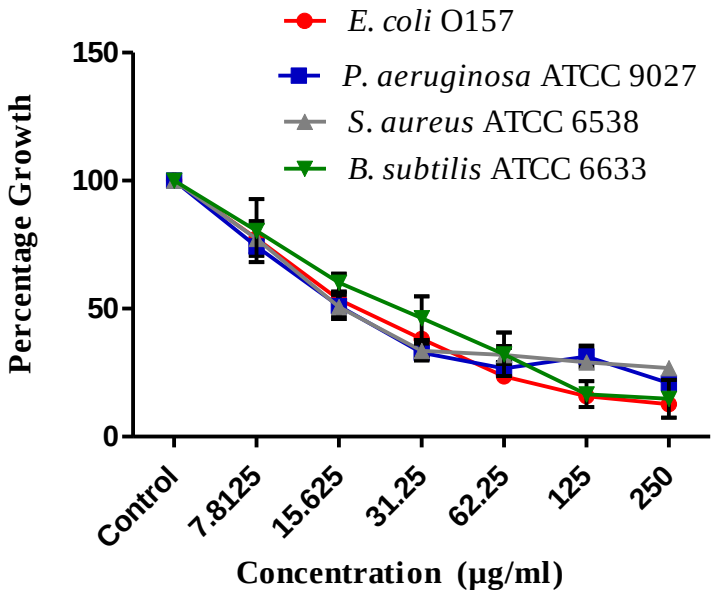

Figure 1. The concentration-dependent activity curve of microbial strains treated with acetone extract of *Opioro* starting from the control (100%). Values in the figure were analysed with two-way ANOVA and then expressed as mean±standard deviation (SD) of three replicates.

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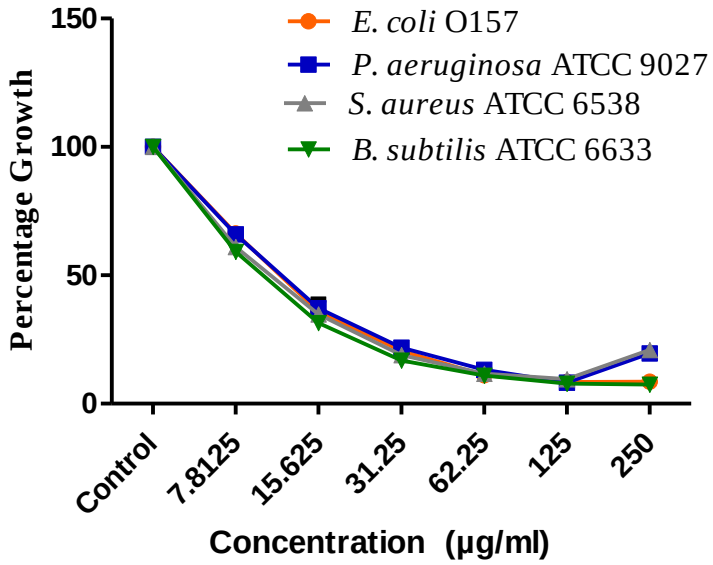

Figure 2. The concentration-dependent activity curve of microbial strains treated with ethanolic extract of *Opioro* starting from the control (100%). Values in the figure were analysed with two-way ANOVA and then expressed as mean±standard deviation (SD) of three replicates.

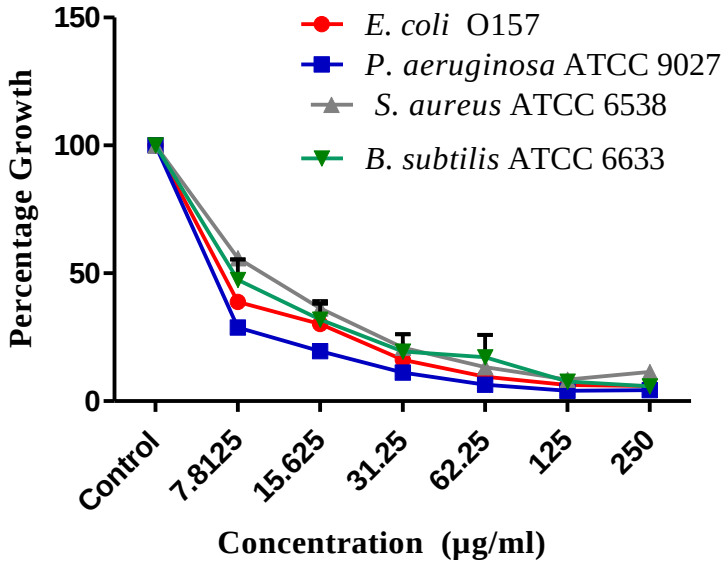

Figure 3. The concentration-dependent activity curve of microbial strains treated with acetone extract of *Julie* starting from the control (100%). Values in the figure were analysed with two-way ANOVA and then expressed as mean±standard deviation (SD) of three replicates.

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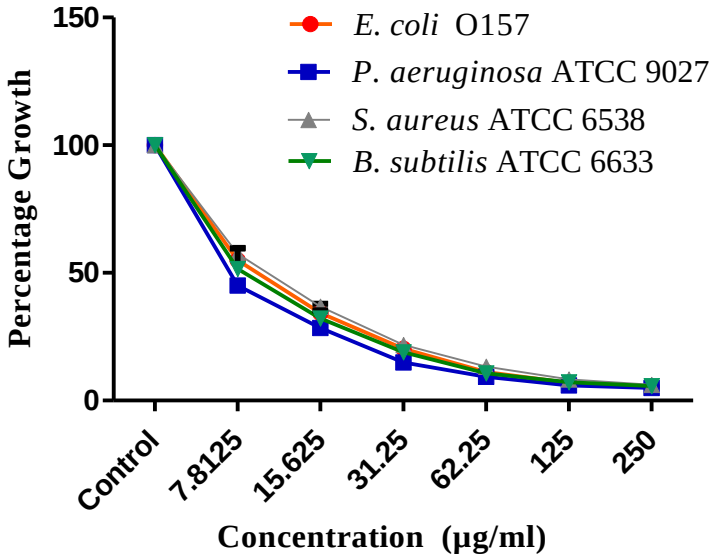

Figure 4. The concentration-dependent activity curve of microbial strains treated with ethanolic extract of *Julie* starting from the control (100%). Values in the figure were analysed with two-way ANOVA and then expressed as mean±standard deviation (SD) of three replicates.

4. DISCUSSION

Phytochemical Test

Phytochemicals are natural components located in the leaves, stem bark, fruits and seeds of medicinal plants responsible for protecting the entire plants against potential pathogens which are replicated when these plant parts are consumed as foods. From the result obtained, the total phenolics, total tannins, total flavonoids, total alkaloids and total saponins of the two varieties were significantly different. The total phenolics, flavonoids and saponins are known to be effective in the management of diabetes, high blood pressure, oxidative stress and over weight (Irondi et al., 2018). In addition to earlier benefits of other phytochemicals, tannins are also responsible for protecting the body against haemorrhage (Sharma et al, 2019).

Antioxidant Test

The antioxidant of *Julie* and *Opioro* varieties were studied using DPPH, ABTS and $FeCl_3$. The DPPH* scavenging assay examines the hydrogen donating ability of the extracts to highly oxidative compound of DPPH (2, 2 diphenyl-1-picrylhydrazyl). The $ABTS^{*+}$ (2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) scavenging assay measures the antioxidant capacities of natural and synthetic compounds with a strong reducing ability while $FeCl_3$ is a strong reducing agent that becomes oxidized to ferric ion or its derivative molecules by oxidizing agent (Irondi et al., 2018).

From the result, the ABTS assay and reducing power of *Julie* were seen to be significantly different from *Opioro*. These higher values of *Julie* could be attributed to the presence of high phenolic compounds, saponins and tannin (Abdel-Aty et al., 2018) which are strong electron donors. The SC_{50} values of *Julie* and *Opioro* in this result were not significantly different, but they are higher than the values obtained from *Sherri*, *Ogbomoso* and *Elemi* varieties in Irondi et al. (2018). The appreciable amount of SC_{50} indicates that *Opioro* and *Julie* extracts could be capable of preventing ageing, cancer, cardiovascular and neurological diseases (Bagchi et al., 2000).

Antibacterial activity

Figures 1–4 show the concentration-dependent activities of acetone and ethanolic extracts of *Julie* and *Opioro* against *E. coli* O157, *P. aeruginosa* ATCC 9027, *S. aureus* ATCC 6538 and *B. subtilis* ATCC 6633 strains. From the Figures, the concentrations of acetone and ethanolic extracts considerably produced decline in bacterial growth. In Figures 1 and 3, the acetone extracts of *Opioro* and *Julie* produced appreciable decrease in the population of the bacterial strains indicated by the sharp decline in the curves. This is due to the ability of acetone in dissolving lipophilic and hydrophilic components of the two seed kernels making it a better solvent than

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ethanol. This assertion is in agreement with Eloff (2019). At low concentration (7.8125 to 62.25 µg/ml), *E. coli* O157, *S. aureus* ATCC 6538 and *P. aeruginosa* ATCC 9027 were susceptible to acetone extract of *Julie* while *S. aureus* ATCC 6538 and *P. aeruginosa* ATCC 9027 were susceptible between 32.25 and 62.25 µg/ml. The high antibacterial activity exhibited by *Julie* extract could be attributed to the elevated values of tannins, saponins and flavonoids (Aguoru *et al*., 2017).

In Figure 2, the ethanolic extracts of *Opioro* at 125µg/ml substantially inhibited the bacterial strains, but, beyond this concentration, *S. aureus* ATCC 6538 and *P. aeruginosa* ATCC 9027 curves rose upward. The sharp rise in the curve is as a result of paradoxical effect, produced by sharp resistance at that concentration. This is an indication that the maximum kill rate of the extract has been reached at that point (125µg/ml) and further increase in concentration is ineffective on the bacterial strains (Gilmore et al., 2011).

Minimum inhibitory concentration (MIC)

The MIC is the lowest concentration of any antibiotic that inhibits the visible growth of microorganisms. From Table 3, the minimum inhibitory concentration results indicate that the antibacterial activities of the seed kernel extracts were concentration dependent. Acetone extract of *Julie* produced the lowest MIC range (7.8125 to 62.25 µg/ml) for *E. coli* O157, *B. subtilis*, *S. aureus* ATCC 6538 and *P. aeruginosa* ATCC 9027 while the MIC values of ethanolic extract of *Opioro* were relatively high (125 µg/ml) for all the bacterial strains when compared with the acetone extracts. The MIC values (62.25 to 125 µg/ml) of ethanolic *Julie* extracts for all the microorganisms clearly suggests that *Julie* could be effectively beneficial than *Opioro* in the treatment of diseases. Generally, *E. coli* O157 and *P. aeruginosa* ATCC 9027 were the most susceptible in all the treatment than the typed Gram-positive strains. The susceptibility of these Gram-negative bacteria could be due to their structural difference (Mutua et al., 2016). The cell walls of Gram-negative bacteria are surrounded by thin peptidoglycan layer making them the most susceptible. Abdalla et al. (2007) also reported that the susceptibility of Gram-negative bacteria is contributed by the phytochemicals, fatty acid and fatty acid derivatives in the seed kernels of mangoes.

5. CONCLUSION

The study conducted showed that *Opioro* and *Julie* varieties are rich in phytoconstituents, which contribute to their high antibacterial activity. Their antioxidant properties are efficient in protecting the body against oxidative stress and chronic diseases.

Acknowledgments

The authors are grateful to the department of microbiology, Abia State University, Uturu, Nigeria for the opportunity to work in their laboratory. We are thankful to the Chief Molecular Scientist, Virology and Molecular Department, University of Benin Teaching Hospital (UBTH), Edo State, Nigeria for providing the laboratory for the conduct of the antibacterial test.

Authors' Contributions

Kingsley C. Nwachukwu performed the experiment and the statistical analysis and wrote the first draft. Ositadinma C. Ugbogu proofread the manuscript and harmonized the paper.

Funding

This study has not received any external funding.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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